GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL [ ] REVISION NO. _____

Project No./(Center No.) G-33-H10 Q5170-0A0 ~~GTRC~~/GIT DATE 6 / 24 / 87

Project Director: Dr. Sheldon W. May; Dr. R. H. Felton ~~School/Lab~~ Chemistry

Sponsor: DHHS/PHS/NIH/National Institute of General Medical Sciences

Agreement No.: Grant No. 5 R01 GM23474-10

Award Period: From 4/1/87 To 3/31/89 (Performance) 6/30/88 Reports

Sponsor Amount:	New With This Change	Total to Date
Contract Value: $		$ 183,980
Funded: $		$ 183,980

Cost Sharing No./(Center No.) _____ Cost Sharing: $ _____

Title: Non-Heme Metallo Oxygenase Catalysts

ADMINISTRATIVE DATA OCA Contact E. Faith Gleason

1) Sponsor Technical Contact:

Dr. Warren Jones (301) 496-7621
Office of Associate Director Program Activities
NIGMS

2) Sponsor Issuing Office:

Linda Roberts/Lucy Clark (301) 496-7746
Grants Management Office
Office Assoc. Director Program Activities
NIGMS

Military Security Classification: N/A
(or) Company/Industrial Proprietary: _____

ONR Resident Rep. is ACO: _____ Yes X No
Defense Priority Rating: _____

RESTRICTIONS

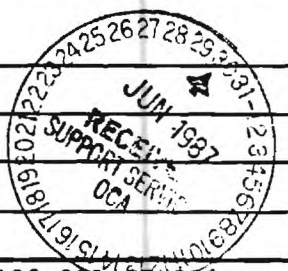
See Attached _____ Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval — Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT. Note: No equipment may be purchased during the final 6 months of the Grant project period - in this case, after 9/30/87.

COMMENTS:

Continuation of G-33-H09 which may be terminated.



SPONSOR'S I.D. NO. 02.108.001.87.016

COPIES TO:

Project Director
Research Administrative Network
Research Property Management
Accounting
Procurement/GTRI Supply Services
Research Security Services
Contract Support Div.(OCA)(2)
Research Communications
GTRC
Library
Project File
Other

12 / 88

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Date 10/4/89

oject No. G-33-H10 Center No. Q5170-0A0

oject Director S. W. May School/Lab Chemistry

onsor DHHS/PHS/NIH

ntract/Grant No. 5 R01-GM23474-10 GTRC ____ GIT XX

ime Contract No. N/A

tle Non-Heme Metallo Oxgenase Catalysis

fective Completion Date 3/31/89 (Performance) 6/30/89 (Reports)

oseout Actions Required:

__ None
__ Final Invoice or Copy of Last Invoice
__ Final Report of Inventions and/or Subcontracts - Patent questionnaire sent to P/I
__ Government Property Inventory & Related Certificate
__ Classified Material Certificate
__ Release and Assignment
__ Other

cludes Subproject No(s).

bproject Under Main Project No.

ntinues Project No. Continued by Project No.

stribution:

__ Project Director
__ Administrative Network
__ Accounting
__ Procurement/GTRI Supply Services
__ Research Property Management
__ Research Security Services

X Reports Coordinator (OCA)
__ GTRC
X Project File
Z Contract Support Division (OCA)
__ Other

5RO1 GM23474
FINAL REPORT

Non-Heme Metallo Oxygenase Catalysis
RO1 GM23474
4/1/84 - 3/31/89

Sheldon W. May, PI
School of Chemistry and Biochemistry
Georgia Institute of Technology
Atlanta, GA 30332

Summary Statement

Non-heme metallo oxygenases represent more than 80% of all known dioxygenases and a large number of monooxygenases, and thus a definition of the molecular basis of their catalytic action is highly relevant to many key biological processes. In this program we analyzed the involvement of non-heme iron in the catalytic pathway of the bacterial dioxygenase, protocatechuate-3,4-dioxygenase (PCD), and carried out comparative studies with the mamalian non-heme iron monooxygenase, liver phenylalanine hydroxylase (PAH), the mamalian coppper monooxygenase, dopamine-B-monooxygenase (DBM), and the bacterial non-heme iron monooxygenase, P. oleovorans epoxidase/hydroxylase (POEH).

A protocol was developed to monitor ESO_2 formation and decay under varying conditions of solvent and concentrations of reactants. The exceedingly high amounts of enzyme required for EXAFS is always a problem which must be overcome, but in this case the problem was confounded by the fact that a buildup of ESO_2 of 2-3 mM would be required for accurate EXAFS analysis, which is far in excess of the total solubility of oxygen in water at room temperature. After many trials, procedures were worked out to buildup ESO_2 under hyperbaric oxygen conditions (3-4 atm.) at 4DoUC, and to rapidly trap this species by freezing at -80 degrees. Kinetic methods were worked out to measure the germane rate constants and monitor formation and decomposition of the transients, in order to assure agreement with values previously determined by steady state and stopped flow analyses. Laborious, large-scale isolations were carried out to provide sufficient enzyme for these studies and for preparation of the sample which was actually subjected to EXAFS analysis.

"ESO_2" was trapped in an EXAFS cell at cryogenic temperature and its optical spectrum measured immediately prior to and following the EXAFS data collection by means of a specially constructed double beam fiber optic spectrometer. The spectra showed some alteration due to irradiation, which is plausibly assigned to ozinide formation in the O_2-rich sample. Upon thawing and further reaction, the characteristic ES spectrum was observed. This confirms that, upon thawing, the expected turnover of "ESO_2" indeed occurred, thus depeleting oxygen and trapping the PCD as ES due to the prsence of excess substrate. Since the solution was frozen, care was taken to move the sample slightly during data collection to reduce or remove Bragg peak scattering. Preliminary analyses showed the following: (1) loss of chelation by substrate or product, (2) a six-coordinate iron, similar to ES but in contrast to native PCD, (3) strong disorder of the first-shell relative to E or ES, (4) retention of histidyl ligation. First-shell analyses on E, ES, EI, and

complexes with transition state analog is complete. The iron in native PCD and its complexes with 3-chloro-hydroxybenzoate (I) or TSA (TSA = 2-hydoxyisoniciotinic acid N-oxide) is five coordinate; in contrast, the iron in ES (S = protocatechuate, 5-bromoprotocatechuate, or dihydroxyphenylpropionate) is six coordinate. Average first-shell distances in E, ES, and EI are about 2.00 A, while E.TSA complexes possess average distances of 2.05 A. Excepting the anomalous EI complex, all species show two histidyl ligands at the active site.

Other experiments in this phase of the program included preparation of crystals of PCD for solid state EXAFS measurements as a prelude to experiments were S and/or I will be diffused directly into such crystals. Also, careful reconstitution experiments on transferrin species were carried out in order to resolve some inconsistencies in our initial EXAFS reults with this protein.

Very good progress was made in the preparation and characterization of modified phenylalanines and pterins. Alternate catalytic competencies for PAH were investigated using our novel substrate analogs, vinyl-Phe and pMeS-Phe.. A prototype of another novel class of compounds was prepared as a potential mechanism-based inhibitor for PAH. The p-pyridine analog of Phe has been prepared and characterized. If PAH is capable of oxygenating such compounds to the corresponding pyridine N-oxides, these should be potent ligands for the Fe and may be excellent inhibitors. Moreover, the N-oxides are very useful EXAFS probes in investigating the question of whether the Fe is actually participating in substrate oxygenation.

Publications

"Structure and EXAFS of Diaquatetrakis(imidazole)cobalt(II) Dichloride", L.R. Furenlid, D.G. Vandervear, and R.H. Felton Acta Cryst. C.\42\806, 1986

"EXAFS of Non-Heme Iron Containing Proteins", P.A. Morris, Ph.D. Thesis, Georgia Institute of Technology, 1986.

"EXAFS of an Enzyme Reaction Transient: The ESO2 of Protocatechuate 3,4-dioxygenase", Fed. Proc. 46, 2261 (1987)

"EXAFS of Non-Heme Iron Proteins", R. H. Felton, L. R. Furenlid, P. A. Mprris, S. W. May, and E. A. Stern, Ann. Rep. Nat. Synchrotron.Light Source,(1986).

"EXAFS of Non-Heme Iron Proteins and Metal Imidazole Complexes," R. H. Felton, L. R. Furenlid, S.W. May, P. A. Morris, and E. A. Stern, Ann. Rep. Nat. Synchrotron.Light Source, 173-175 (1985).

"Solvent Proton Magnetic Resonance Dispersion in Protocatechuate 3,4-Dioxygenase and Complexes with 3-Halohydroxybenzoate Inhibitors," R. H. Felton, S. L. Gordon, A. L. Sowell, and S. W. May, Biochemistry, 22, 5331-5340 (1984).